Biological control of dog-strangling vine: will *Hypena opulenta* ever eat enough?

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photo credit: Jim des Rivières

These are the slides for my Ottawa Entomology Club presentation on March 18, 2021.

Dog-strangling vine (DSV)
or pale swallow-wort
Vincetoxicum rossicum
(Kleopow) Barbaricz

- Dogbane family (Apocynaceae)
- Native to Ukraine
- In North America since late 1800s
- Herbaceous vine
- Invasive in both open habitats and forest understorey

Quick introduction to dog-strangling vine, also known as pale-swallowwort, Vincetoxicum rossicum. We also have a closely related species, black swallowwort, but only a few small patches of it here in Ottawa. Black swallowwort is much more common in the New England states. Both species have been in NA since the late 1800s, early 1900s, and it took them a while to become invasive.

Dog-strangling vine (DSV)
or pale swallow-wort
Vincetoxicum rossicum
(Kleopow) Barbaricz

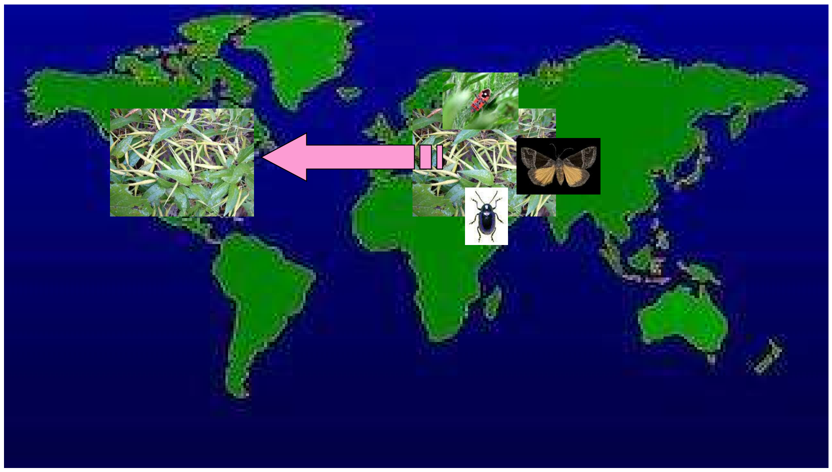
- Self pollinates
- Polyembryonic seeds
- High germination rate
- High seedling survival
- Big investment in roots
- Facilitates own growth
- Almost no herbivory in North America

Has a lot of characteristics that make it a good invader.

Control of DSV is very difficult

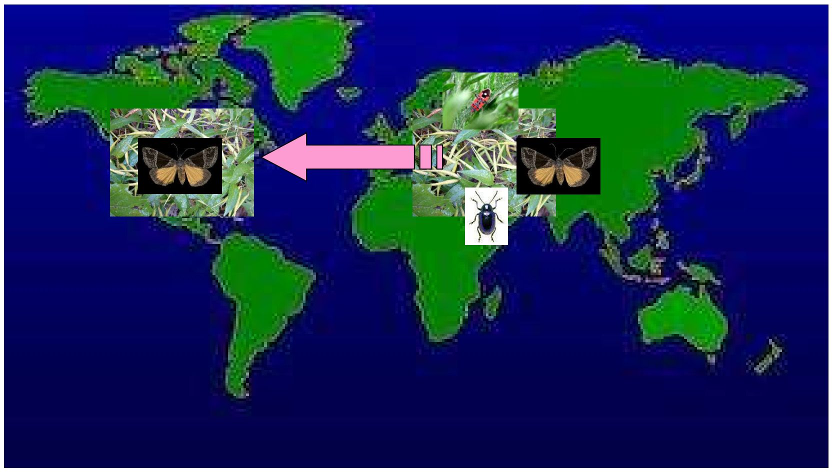
Dog-strangling vine or pale swallowwort *Vincetoxicum rossicum* at the Fletcher Wildlife garden

Classical Biological Control and the Enemy Release Hypothesis



Classical Biological Control has as its underlying assumption the Enemy release hypothesis. Non-native species come to North America leaving their natural enemies, in the case of plants this would be herbivores, behind in their place of origin. Not having any enemies, they come to dominate the landscape. Of course, some alien plants are fed upon by native North American herbivores; these tend to become benign members of their new communities. A smaller number, like DSV, have toxic defensive chemicals that North American herbivores are not adapted to eat. These are typically the ones that become highly invasive.
https://www.researchgate.net/publication/6651506_Novel_chemistry_of_invasive_exotic_plants

Classical Biological Control and the Enemy Release Hypothesis



In classical weed biological control programs, we bring the herbivores from original range of the invasive pest and release them in the new range so they will hopefully knock back their invasive host plants. Won't drive their native hosts to extinction, but rather the goal is to turn them into a minor member of the plant community.

What is the risk of introducing another non-native organism to control a non-native pest?

Wikimedia Commons

We can't talk about biocontrol without addressing the risks of introducing one non-native organism to combat another. And here's that well-used cane toad photo again. The poster child for biological control gone wrong. It was introduced into Australia in 1935 to control beetles in sugar cane and become horribly invasive itself.

Target: Musk thistle *Carduus nutans*
Photo credit: Ann Burgess
www.geograph.org.uk

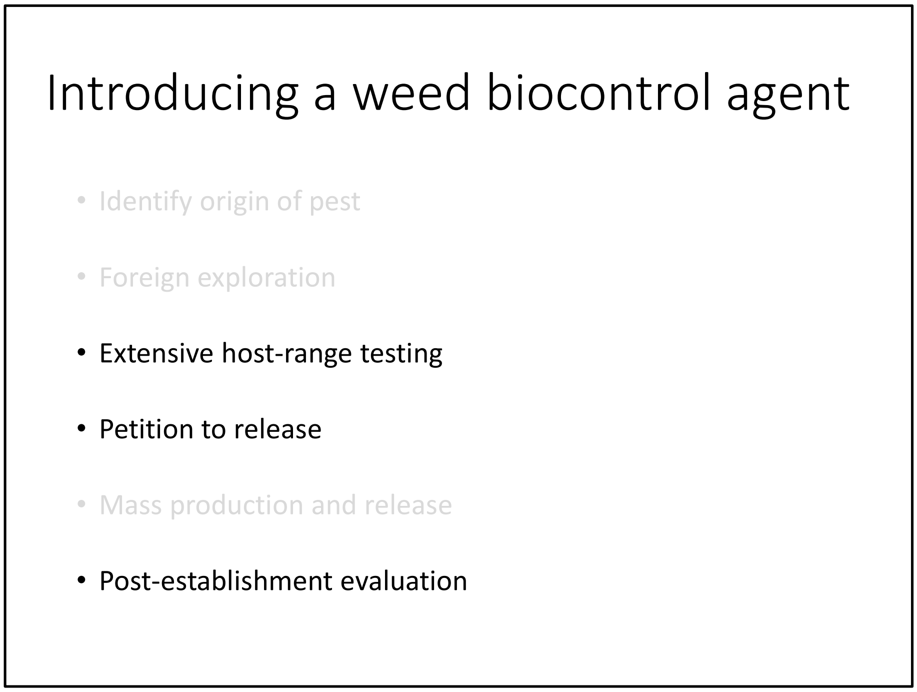
Rhinocyllus conicus
The "cane toad" of weed biocontrol
Photo credit: Udo Schmidt
Wikimedia Commons

The cane toad of weed biocontrol is Rhinocyllus conicus. It was introduced to NA in 1969. Very effective biocontrol agent of musk thistles, but it causes collateral damage on non-target native thistles, some of which are endangered species. Biocontrol mistakes, such as the cane toad and Rhinocyllus conicus, are the reason that we now have such stringent guidelines for releasing agents.

Introducing a weed biocontrol agent

- Identify origin of pest
- Foreign exploration
- Extensive host-range testing
- Petition to release
- Mass production and release
- Post-establishment evaluation

Introducing a biocontrol agent is a long careful process now often taking up to 10 years from foreign exploration to release. Great care taken at the host-range testing (what else could it eat?) Petitioning to release something is akin to approving a vaccine…candidates are carefully reviewed by a panel of scientists and government agencies with different perspectives.



I'm going to talk briefly about the host-range testing mainly to convince folks about the very very low risk posed by the release of Hypena into the Canada and the US. Then I'll talk about some issues that were raised in the US regarding the petition to release there which provided the incentive for some student projects post-establishment. I won't have anything to say about mass production because basically that's not happening…Hypena has proven quite finicky to rear in the lab, which is why we have to turn down requests for purchasing them.

Hypena opulenta

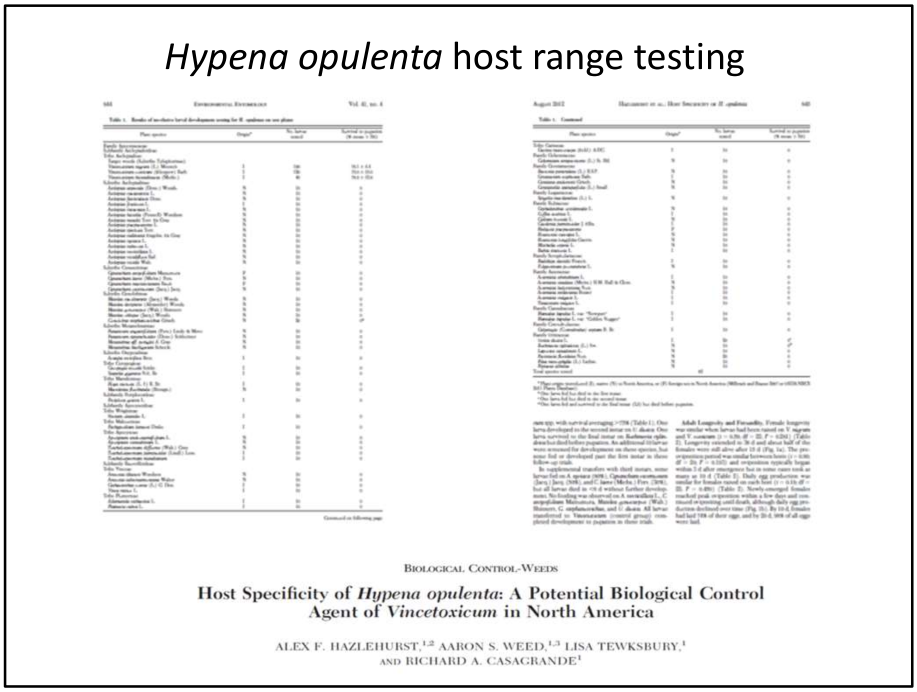
Foreign exploration, host-range testing, petition to release:
University of Rhode Island Biological Control Lab

Photo: Todd McLeish
RI Monthly, September 20, 2016

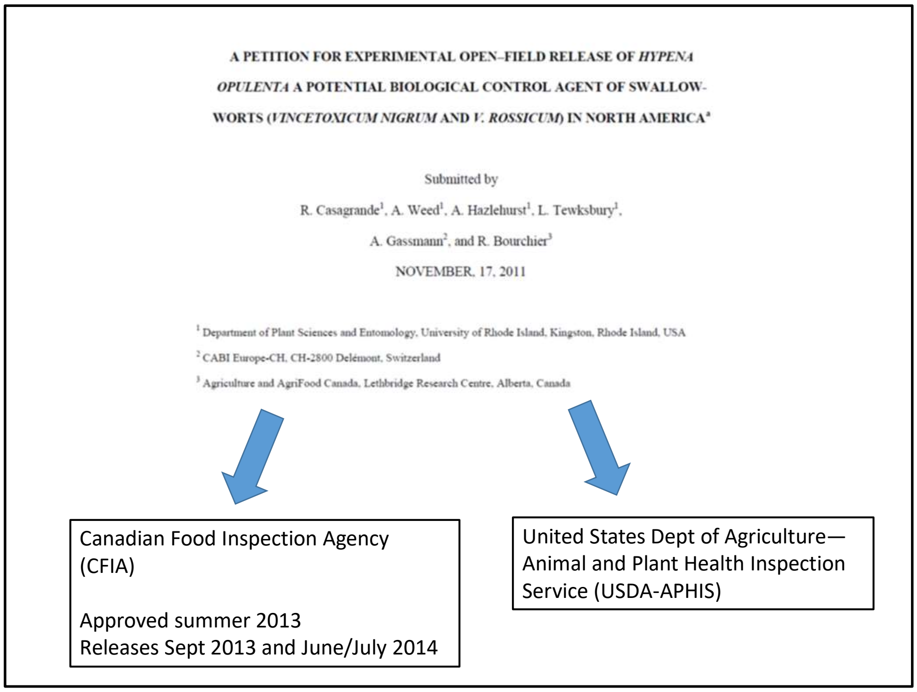
https://www.aaronsweed.com/

Lisa Tewksbury, Dick Casagrande and Aaron Weed

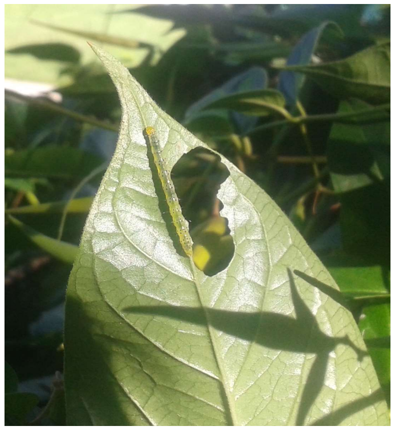
First I want to give a shout out to the folks that did all the hard work prior to release, my good colleagues from the University of RI…



Can't read this …but this is to show you that a lot of plant species were tested to make sure that Hypena couldn't eat them…82 species in all. Third column is survival of the caterpillars on a given host plant species, and mostly what you see is a string of zeros, except for the target host plants (top left): Vincetoxicum nigrum, Vincetoxicum rossicum and Vincetoxicum hirundinaria, a European species that is not found in North America



Based on these promising results from the host-range testing, petition to release Hypena was submitted to CFIA (Canada) and USDA (APHIS) in 2011. The Canadian petition was approved in the summer of 2013. A release took place at the Experimental Farm in September of 2013, and again in June/July of 2014.

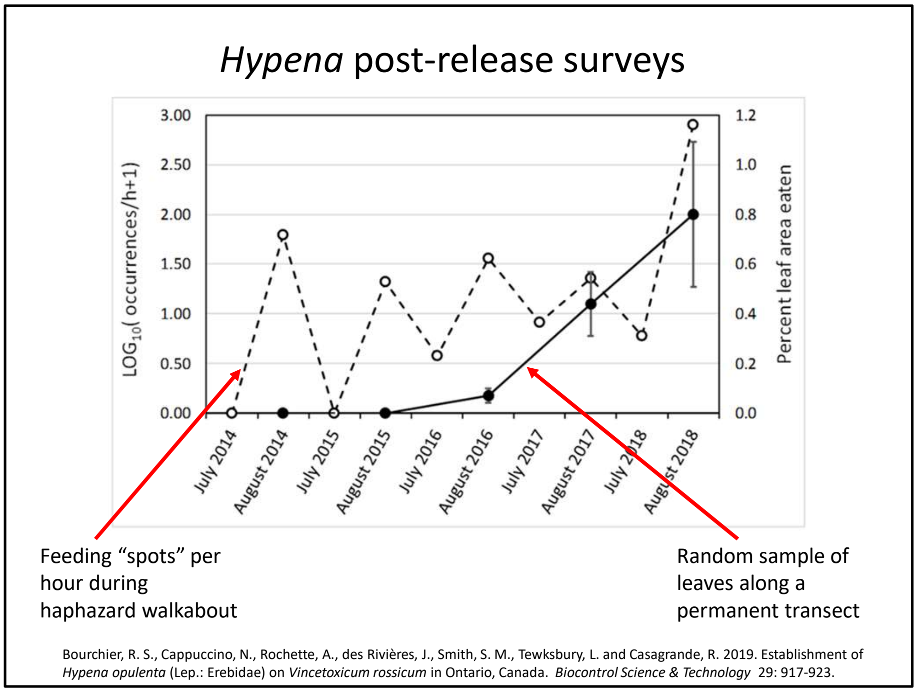


Successful second generation,
August 2014

Emergence in overwintering cage 2015

Individual caught at
light trap, June 2016

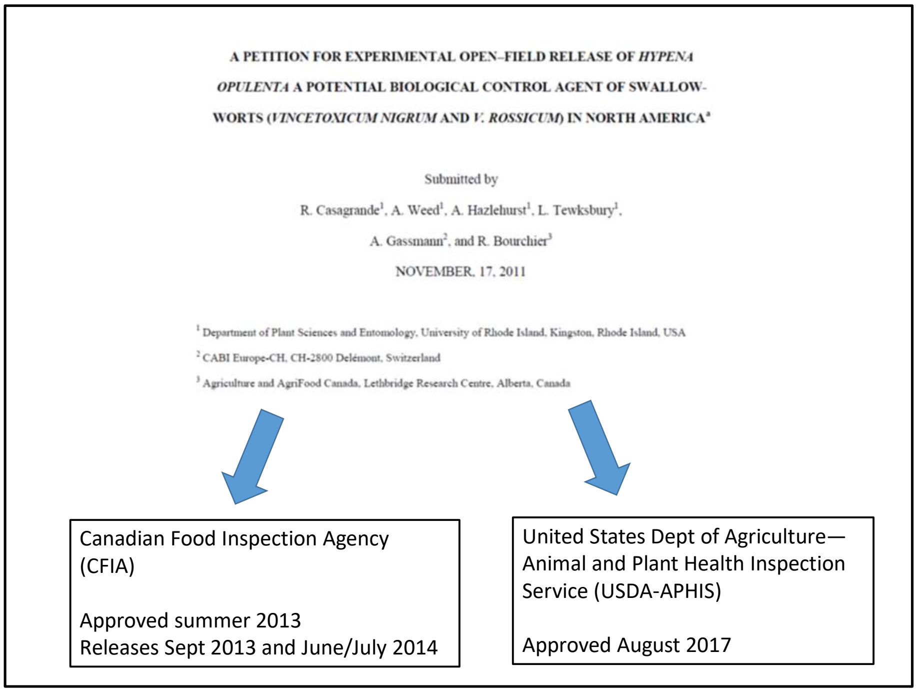
In August 2014 we saw a new generation of caterpillars. In 2015, we saw emergence from pupae that had overwintered in a field cage, with the netting removed to allow for snow cover and then replaced after snow melt. In June 2016 we light captured this individual at a light trap. Establishment of a local population was confirmed.



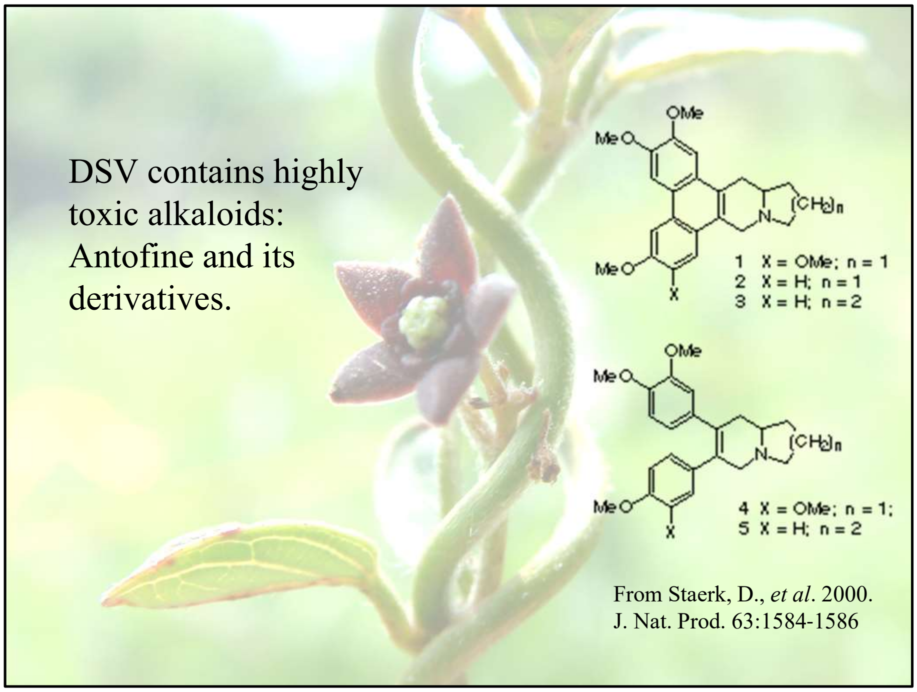
Over four years we saw undeniable population growth. Hypena at low numbers is very difficult to count. The larvae are green and hide, very hard to see. So we looked for feeding damage instead. We had two ways of doing this: feeding spots during a timed walkabout and random samples of leaves for damage along a permanent transect. Feeding spots were done twice a year corresponding to the two generations. So you see the zig zag of a smaller or undetectable first generation followed by more feeding spots in the second generation. The August 2018 numbers are actually a big increase because the scale is logarithmic. The random leaf sample was done in late August of each year. The increase is steady, but the percentage leaf damage is very very low, less than 1% of leaf area eaten.

August 2018 in one hedgerow at the Experimental Farm

However in August 2018 in one hedgerow at the Experimental Farm we began to see substantial damage…even some plants that were completely defoliated.

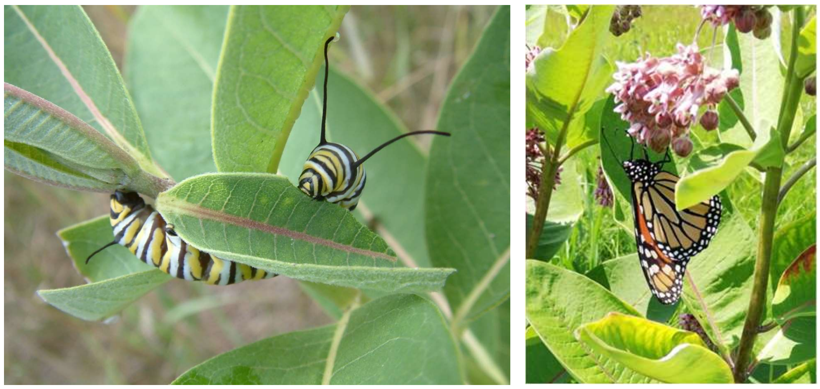


Meanwhile, at the USDA, the petition was stuck in limbo for a few years. Reviewers from Fish & Wildlife Service requested an overview of anticipated ecological effects beyond the usual "will they feed on non-target plants?". They raised a number of questions about the possible impact of a Hypena release the other species in the system that might eat Hypena. They suggested that since Hypena feeds on a toxic host plant, it might sequester those toxins for its own defense and be dangerous for predators such as birds and bats.



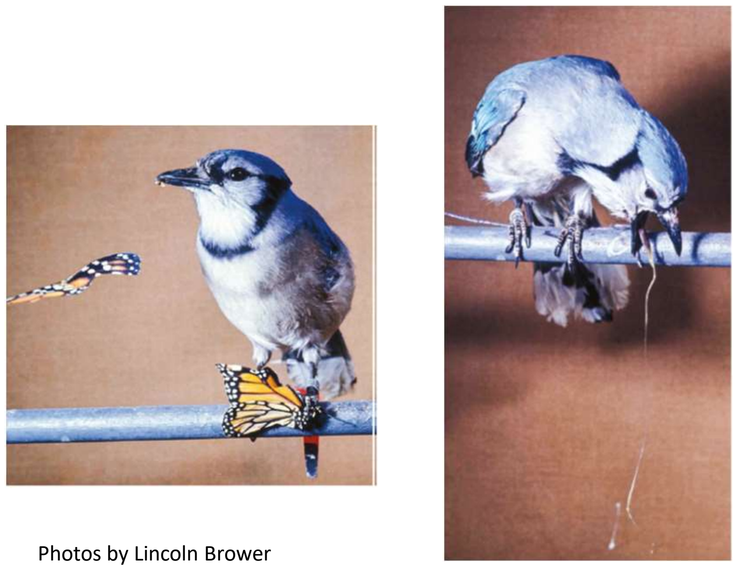
Dog-strangling vine is indeed a highly toxic plant. It contains toxic alkaloids that are unusual in the dogbane family. These alkaloids have attracted quite a bit of attention from plant chemists because they are highly active: they have anti-fungal, anti-bacterial, anti-cancer properties. There is some older literature reporting the presence of glycosides in DSV as well, the heart toxins that we associate with milkweeds.

The US Fish and Wildlife folks were concerned that Hypena caterpillars be able to sequester these toxic compounds for their own defense. Would this possibly make them deadly to their own predators? (birds feeding on the larvae and bats on the adults seemed to be the biggest concern of FWS)? This was the first time this question had come up during the review of a biocontrol petition…and it is a fascinating one to think about and argue about. But it’s a question that has a reasonable answer if you think about it from the point of view of an ecologist.



Caterpillar that sequesters toxins from host plant:
monarch *Danaus plexippus*

There are plenty of toxic Lepidoptera already out there…monarchs come to mind.



Photos by Lincoln Brower

https://academic.oup.com/ae/article/63/2/70/3867366?login=true

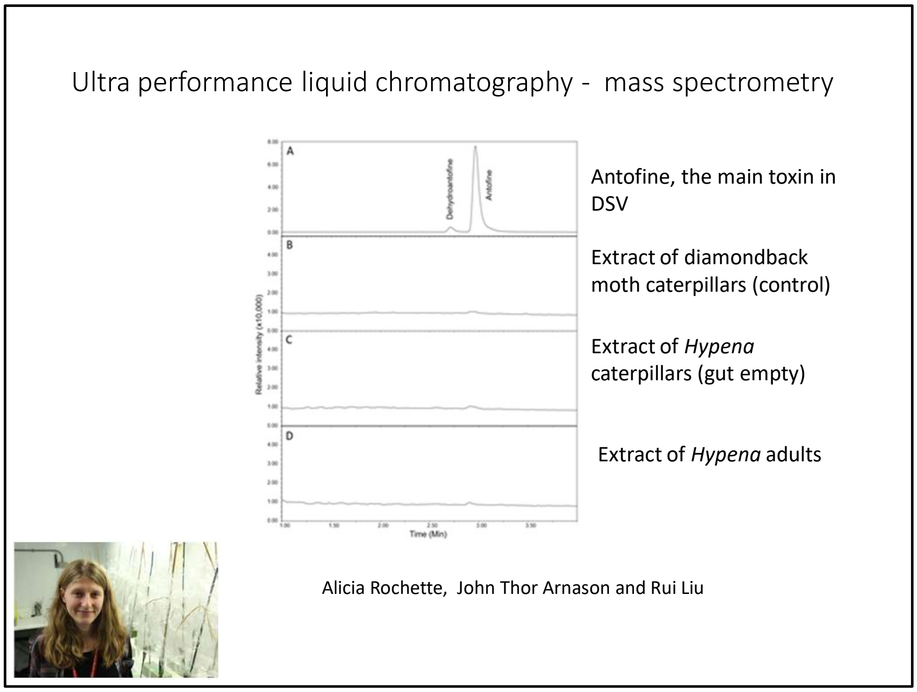
And what happens when a monarch is eaten by a naïve young bird is well documented…it doesn’t die, but it definitely learns to avoid monarchs. It also learns to associate orange and black stripes with a bad experience and will avoid insects with this warning colouration or “aposematism”.

Cinnabar moth caterpillar *Tyria jacobaeae*
Biological control agent of tansy ragwort
Photo: Quartl on Wikimedia Commons

Toadflax brocade moth caterpillar
Calophasia lunula
Biological control agent of toadflax

Toxic biological control agents have been released in the past. Nobody has noticed any negative effects on wildlife, although to be fair, nobody has really looked. Follow up studies on biocontrol agents are almost always about the effect on the target host plant, not about what is eating the agent.

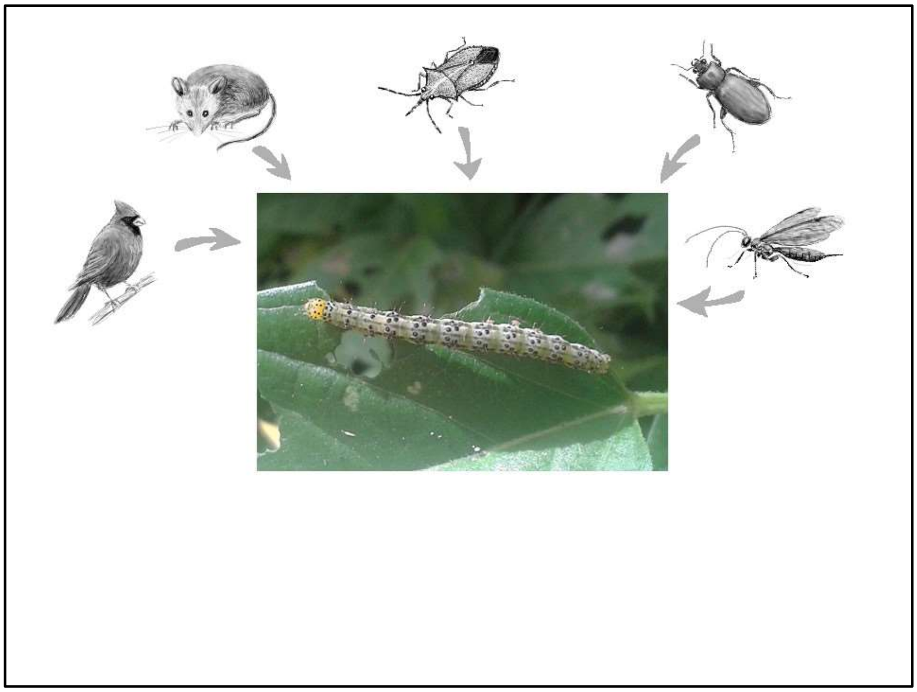
Hypena opulenta is nothing like a toxic caterpillar. It doesn't look or act like a toxic caterpillar with their aposematic colouration. It is green, it hides, does most of its feeding at night, and if you just look at it the wrong way it hurls itself off the plant. Toxic caterpillars are not only boldly coloured, they have bold behaviour. You can pet a monarch and sometimes it doesn't even stop feeding.
So it was suggested to FWS that Hypena was highly unlikely to be toxic…



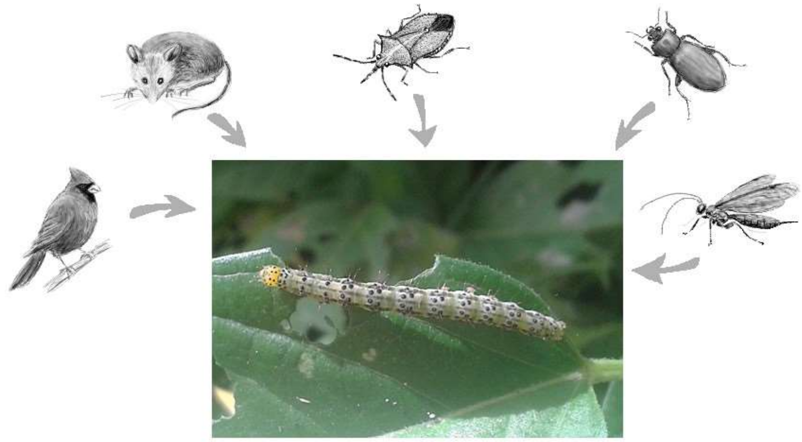
But just to be sure I sent my honours student Alicia Rochette up to Ottawa U to my good colleague Thor Arnason's lab so they could run some of their chemistry magic. No antofine in Hypena. Of course there are other compounds in Vincetoxicum rossicum (most plants have many different defensive compounds), so it is possible that Hypena is sequestering something else.

The argument that Hypena will not pose a danger to its own predators was put to the FWS, and they finally gave the green light for releases in the US in 2017.

Not being toxic might be good from the perspective of easing the collective minds of the FWS, but at first glance, it doesn't' bode well for the prospects of Hypena becoming a successful biocontrol agent.



It means that Hypena is likely to be undefended and it will fall prey to any number of natural enemies in North America—birds, mammals, insect predators and spiders, parasitic wasps—which wouldn't be helpful for its population growth. We basically want Hypena populations to rise to the point at which it has an "outbreak" on its target host, DSV



Hypothesis: Successful biological control agents are the ones that suffer less mortality from natural enemies in their new range

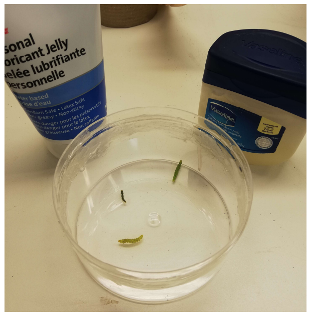
In fact one might apply the enemy-release hypothesis to biological control agents. Agents are brought to North America leaving behind their enemies in their original range. But they will inevitably encounter some native natural enemies here.
One might hypothesise that the successful biocontrol agents are those that suffer less mortality from natural enemies in their new range. Fewer enemies should meant that they can reach high enough densities to significantly damage their host plant. This is an intellectually satisfying hypothesis that could be answered by studying predation and parasitism of biocontrol agents that are established but do not do a good job controlling their host plants and comparing it to predation and parasitism of agents that do a good job (theirs should be lower).
We've been picking away at this for the past few years with small projects that my honours students have done…and what we've found has surprised us… going to start out with bird predation on caterpillars

Bird predation on *Hypena* caterpillars

Honour's student Olivia Nycholat

DIY feeder

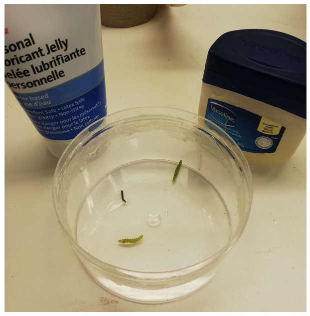
Almost all studies that look at birds eating caterpillars use chicks and do it in the lab. This raises animal care issues and I would have needed an lab appropriate for housing chicks. If we used feeders and presented caterpillars in dishes, and if any supposedly toxic caterpillars were present in nature anyways, then we were fine from an animal care perspective.



Experimenting with different ways to keep the larvae in the dish

Larvae size-matched with control mealworms

Some caterpillars are climbers, so we experimented with different ways to keep them in the dishes. A mix of Vaseline and personal lubricant worked great for all species except Hypena, which is extremely mobile good climber. For Hypena, we solved this problem by using them in their last instar, just before pupation, when they slow down, empty their guts and get ready to pupate—this was the only stage we could keep in the dishes. And the gut emptying is important too, as it means that we are testing birds' response to just the caterpillars, not the caterpillars plus the plant material they have in their guts (and might be able to regurgitate onto an attacking predator)



Experimenting with different ways to keep the larvae in the dish

Larvae size-matched with control mealworms

Hypena and seven other species vs mealworm controls
Mix of choice and no-choice trials
24 hours (morning to morning)
Sample size variable depending on availability

Mealworms were almost always taken, and when they weren't, the other species would still be there as well…we considered that these feeders had not been visited and omitted them from analysis.
Going to summarize the data as overall % taken from the dishes. Caveat…"taken from dishes" doesn't necessarily mean "eaten" since they could have been rejected, but we actually did see some birds eat what was in the dish while at the feeder or fly off and feed it to waiting fledgeling.

Mmm, delicious!

100%

Epiblema scudderiana
Goldenrod gall moth

100%

Vanessa cardui
Painted lady
on bull thistle

100%

Hyles euphorbiae
Spurge sphinx
Biocontrol agent
on cypress spurge

These were the most acceptable species. The first two were not a surprise, we did not expect them to be toxic. I had assumed that the spurge sphinx would be toxic. Its host plant is toxic (phorbol esters, which are toxic irritants). It is aposematically coloured and has bold behaviour. I was holding the sprig of spurge as I took this photo and the caterpillar just kept on eating.

Video: handling *Hyles euphorbiae*

In the video (provided separately on the webpage) the cardinal picks up a caterpillar and the drops it in the dish several times. It then whacks the caterpillar against the side of the dish before eating it. It ate all three caterpillars, and when I looked in the dish there was green caterpillar regurgitant on the sides and bottom of the dish.
The videos are

It turns out that spurge sphinx does not actually sequester the toxins from its host plant in its body tissues. Instead, it regurgitates its gut contents containing phorbol esters, which are toxic and irritant, when grabbed by a bird. The cardinal did the exact right thing by whacking the guts out of this caterpillar!

Meh…

61.5%

Vanessa atalanta
Red admiral
on stinging nettle

51.5%

Bombus mori
Domestic silkworms
from pet store

These two species were eaten about half the time. The silkworms (which were not feeding on mulberry like the ones in the photo which were my personal pets) may have been too large for some of the birds. I would have otherwise expected them to be highly palatable.

Blech!

33.3%

Calophasia lunula
Toadflax brocade moth
Biocontrol agent
on yellow toadflax
Known to sequester
iridoid glycosides

9.68%

Hypena opulenta

0%

Euchaetes egle
Milkweed tussock moth
on common milkweed
Possibly sequesters
cardiac glycosides

Toadflax brocade moth is a biocontrol agent that is known to sequester the iridoid glycosides of its target host. Milkweed tussock moths are thought to be toxic, but there is no real evidence in the literature documenting that. They are aposematic and very hairy, so not surprising that they were not eaten. However, what's going on with Hypena. It looks like it ought to be delicious, but the birds rejected it.

Blech!

33.3%

Calophasia lunula
Toadflax brocade moth
Biocontrol agent
on yellow toadflax
Known to sequester
iridoid glycosides

9.68%

Hypena opulenta

0%

Euchaetes egle
Milkweed tussock moth
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Cardinal rejecting *Hypena opulenta*

Video is provided separately on the webpage showing cardinal eating the last mealworm in the dish and then looking for something else to eat, eyeballing the contents of the dish (three Hypena larvae) from several angles, then flying off, disappointed. (Sorry, I know I shouldn't, but I can't help but ascribe human sentiments to the cardinal!)

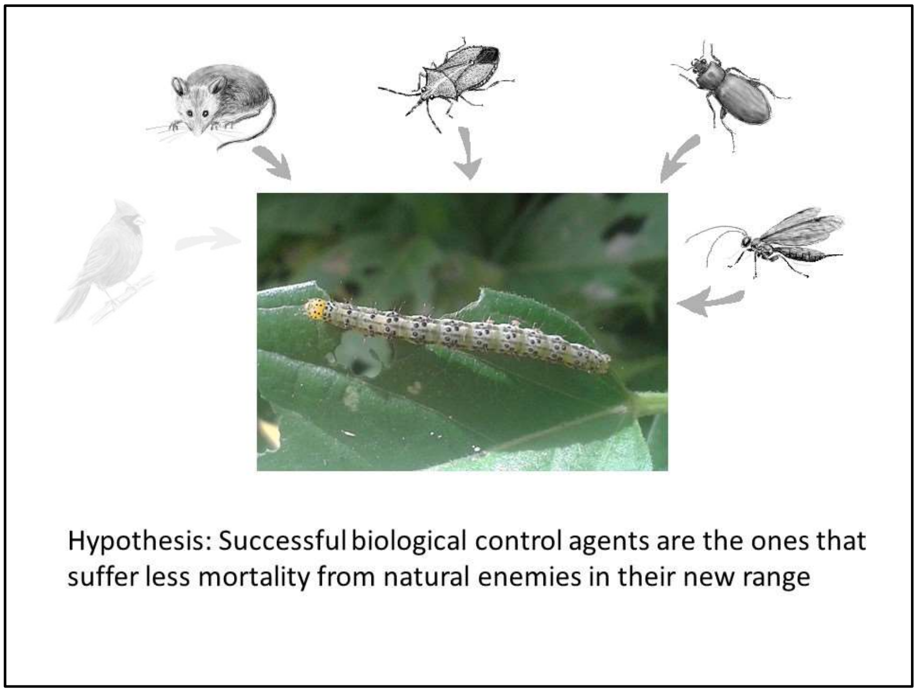
Why were *Hypena* caterpillars rejected?

The cardinal was naïve. That video was taken on the first date Hypena were offered. My house is 7km from the Hypena release site, so it's highly unlikely that the cardinal ever experienced a Hypena caterpillar in the wild.

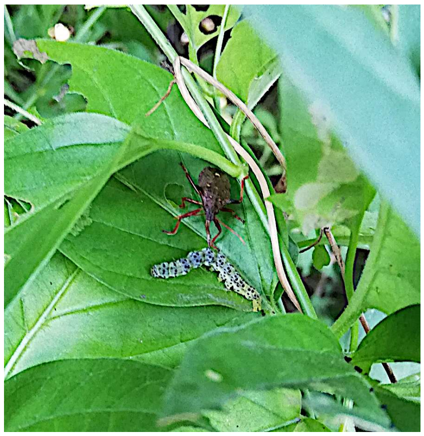
Hypena larvae don't contain the main toxin of DSV (although there might be other toxins such as cardiac glycosides).
Don't regurgitate. Guts are not full of plant material at the stage we tested them (and we saw how cardinal handled the problem of toxic gut contents)
Not aposematic, at least to our eyes. Don't have any audible sounds. Bad breath? Tobacco hornworms exude nicotine from their spiracles and deter spiders, so this is possible, although Hypena don't have a noticeable odor (unlike toadflax brocade moths, which stink)

1) Good from a biocontrol perspective
2) From FWS perspective: birds do the appropriate thing

So this is absolutely great news from a population dynamics perspective…one huge cause of caterpillar mortality, bird predation, is not a factor holding back their population growth! Also, I think the study speaks to the question of the toxic biocontrol agents as bird killers…birds somehow seemed to know what to do.

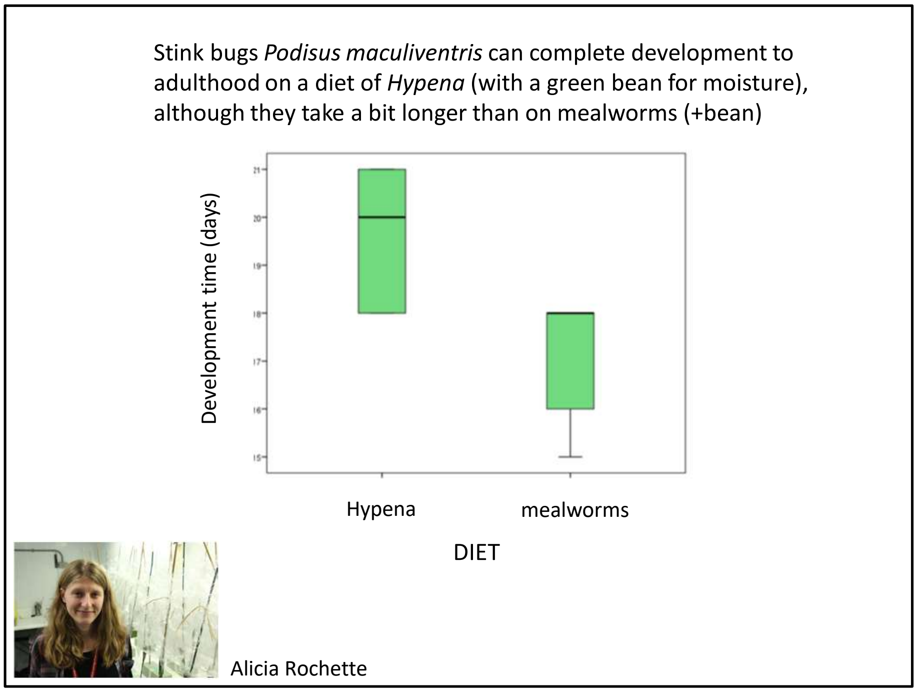


So birds (at least cardinals, chickadees and white-throated sparrows, which were the most abundant birds at our five feeders) do not appear to be a major source of mortality for Hypena. Let's take a look at stinkbugs…they'll eat anything. And ground beetles, which are omnivorous, eating seeds and a wide variety of invertebrates. They might eat a Hypena that leaps off the plant and ends up on the ground.



Stinkbugs *Picromeris bidens* (left) and *Podisus maculiventris* (right)

In fact, the only natural act of predation I have observed on Hypena was this one, by the non-native stinkbug Picromeris bidens. The native spined soldier bug, Podisus maculiventris (right), is well known as a generalist predator with a highly catholic diet. We were able to rear them from second instar nymphs to adulthood on a diet of Hypena.



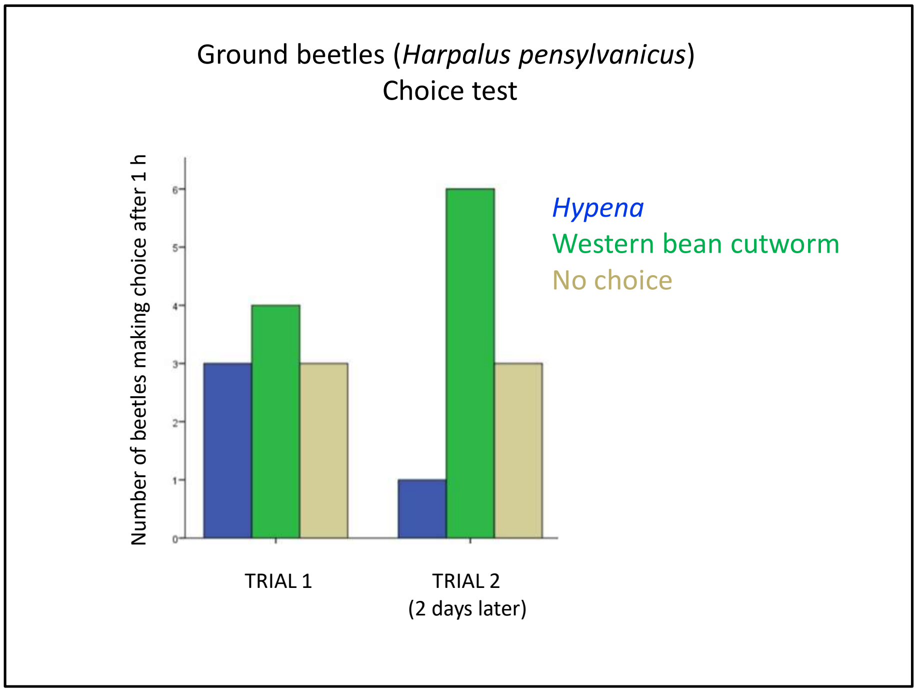
In her honours work, Alicia Rochette showed that stinkbugs can complete development to adulthood on a diet of Hypena. They took a bit more time to reach adult stage than on the control diet (mealworm +greenbean)

Ground beetles (*Harpalus pensylvanicus*)

5 of 7 died after 6 days on a diet of *Hypena*
None died on diet of western bean cutworm (control)

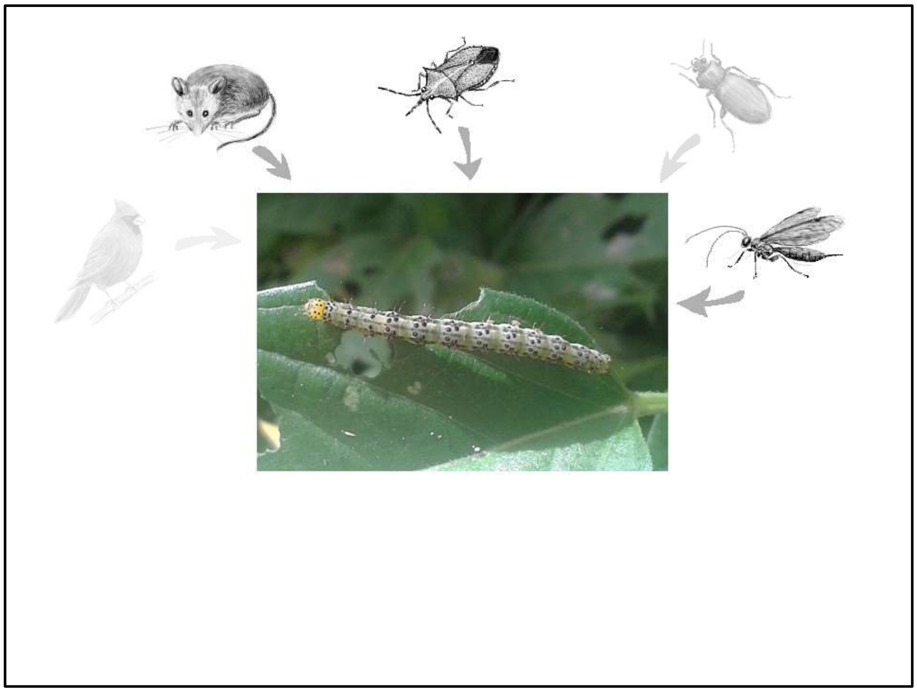
https://agrilifeextension.tamu.edu/

Ground beetles didn't fare as well. On a diet of just Hypena, five of seven died after 6 days. Unlike stinkbugs, which feed selectively on their victim's hemolymph, ground beetles are messy feeders which probably get a mouthful of the caterpillar's gut contents.

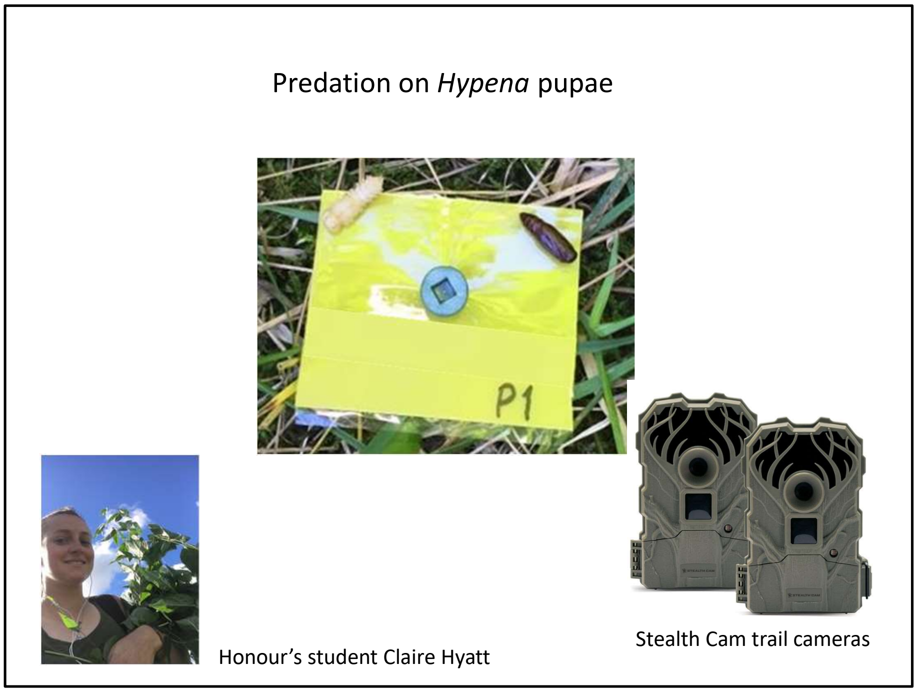


But the ground beetles did show some evidence of avoidance learning. Alicia gave them a choice between Hypena and WBC and watched them for an hour to see which they'd eat. When first given the choice, they showed no preference, but two days later the same beetles seemed to prefer WBC. It's a small sample size, but it looks like the beetles are able to learn.

We really need to repeat this study!



Next, we wanted to take a look at mammal predation on pupae.



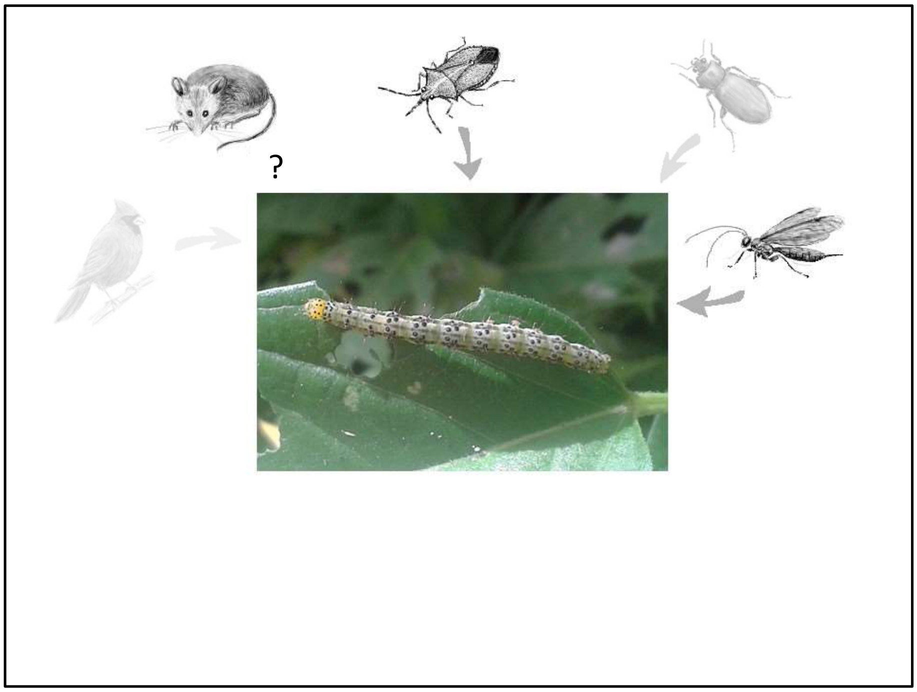
Hypena pupates off the plant in the interface between litter and soil, so they are are subject to predation by ground-dwelling critters. Honours student Claire Hyatt offered Hypena pupae and mealworm pupae to ground-foraging predators on sticky cards anchored into the ground with a deck screw. She set up stealth cams to record what came to the feeding stations.

Visitors to the feeding stations

Hypena: 18/27 = 66.7%
Mealworm: 24/27 = 88.8%

The trail cams captured lots of visitors to the feeding stations…often several different species over the course of a 24-hour period, so it turned out to be impossible to determine who actually ate the pupae. The pupae seemed to be a bit more vulnerable than the larvae with respect to percentage taken.

In real life, however, the pupae would have found a hidey hole to pupate in, and made a loose cocoon, covering themselves with soil and debris, which might afford them some protection from ground-foraging predators.

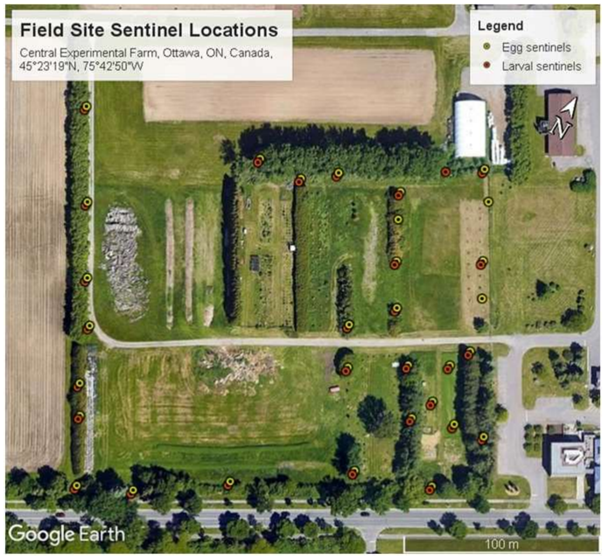


So we’re not really sure how much predation is to be expected by small mammals, although the pupal stage itself seems to be a bit more vulnerable than the larvae.
Finally, we took a look at another of the main sources of mortality for caterpillars: parasitoids. These parasitic wasps lay their eggs inside the caterpillars. When the egg hatches, it devours the caterpillar from the inside out. Many of these wasps are specialized to only parasitize a certain species. Hypena of course left all its specialist parasites in Ukraine, because only clean, unparasitized Hypena individuals were released. But there are some generalist parasitoids as well, so we wanted to see if any of these were attacking our Experimental Farm population of Hypena.
Next up: parasitic wasps (parasitoids). These are wasps (sometimes flies) that lay an egg in the caterpillar. The egg hatches and eats the caterpillar from the inside out. Instead of that caterpillar turning into an adult moth, you get an adult wasp.

Sentinel eggs and larvae for parasitism

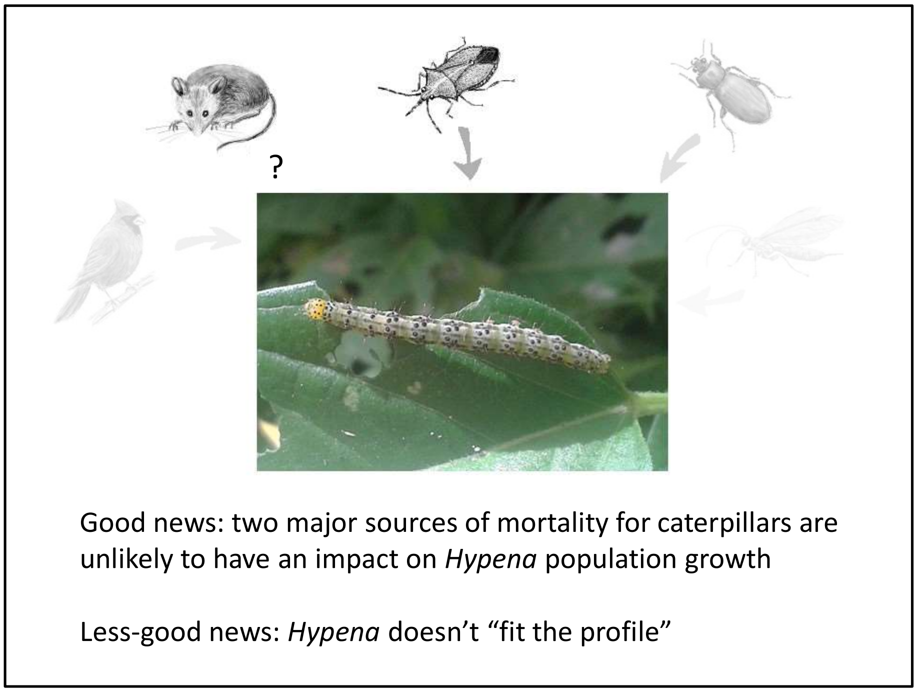
Honour's student Shane Minor

Shane took what is known as a "sentinel" approach: putting out lab-reared eggs and larvae on potted plants, and then bringing them back into the lab to rear out any parasitic wasps that might have attacked them.



Egg parasitism = 0. Larval parasitism = 0.

Shane put out 650 eggs and 200 larvae and got nothing. Which was a bit boring for him, but good news for Hypena. And that is not for lack of availability of generalist parasitoids in the area. Studies on leek moth and diamondback moth have shown that generalist parasitoids are present on the Experimental Farm.

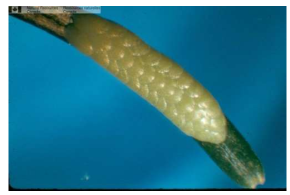


So the good news is that birds and parasitoids are not a major sources of mortality for Hypena. The less-good news is that Hypena doesn't "fit the profile" of an outbreak species, which is what we want it to become.

Uroleucon aphids on goldenrod
(Photo: Wikimedia commons)

Tent caterpillars

Gypsy moth



Spruce budworm egg mass
(photo: Great Lakes Forestry Centre
Insect Production Services)

Hunter, A. E. 1995. Ecology, life history, and phylogeny of outbreak and nonoutbreak species, pp. 41-64. In N. Cappuccino and P. W. Price [eds.], Population dynamics: new approaches and synthesis. Academic Press, New York.

This more pessimistic view actually comes from my PhD thesis work, before I was involved in Biological control. I studied population dynamics of insect herbivores from a more theoretical perspective, using goldenrod herbivores as a model system. Then I worked on spruce budworm for several years. I was interested especially in the question of what traits characterize insect species with outbreak dynamics and the flip side, what keeps some populations at low, apparently stable densities, where they eat only a tiny fraction of the biomass of their host plant. In biocontrol, we want to create outbreaks of the agents. Are there some characteristics that are regularly associated with outbreak species?
My work on goldenrod insects, and a survey of Canadian forest Lepidoptera by Alison Hunter, showed that species that outbreak tend to have at least one life stage where they congregate: laying eggs in masses forming colonies, or feeding communally as larvae. The advantage of this clustering behaviour and how it translates into outbreaks, is that it is thought to allow populations to locally exceed predation pressure by providing a safety-in-numbers.

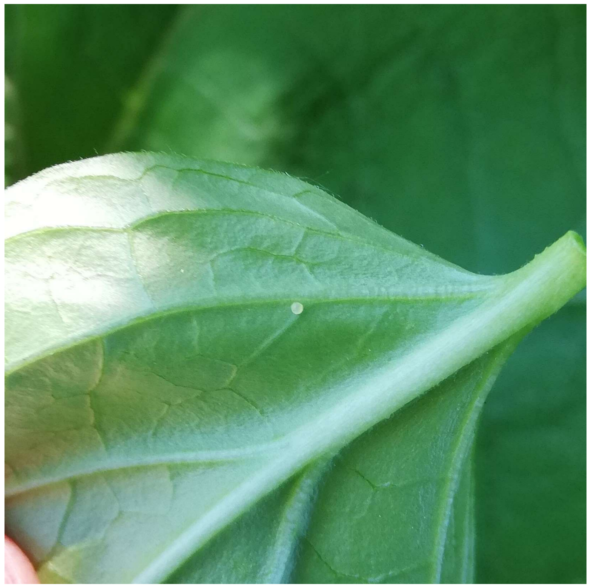
Uroleucon aphids on goldenrod
(Photo: Wikimedia commons)

Brown hooded owlet caterpillar

Luna moth caterpillar

Warty leaf beetle larva

Species that do not outbreak tend to not congregate, they lay their eggs singly, and are more evenly distributed in space. The green goldenrod aphid is related to the red, but does not aggregate or form large colonies and it is very mobile. It has much more “stable” population dynamics than its red cousin. The other three species pictured lay eggs singly and do not outbreak. They are just three examples of many. The majority of insects are not outbreak species; most are relatively uncommon and do not have populations that rise to the level where they eat most of the biomass of their host plants the way gypsy moths or spruce budworms do.

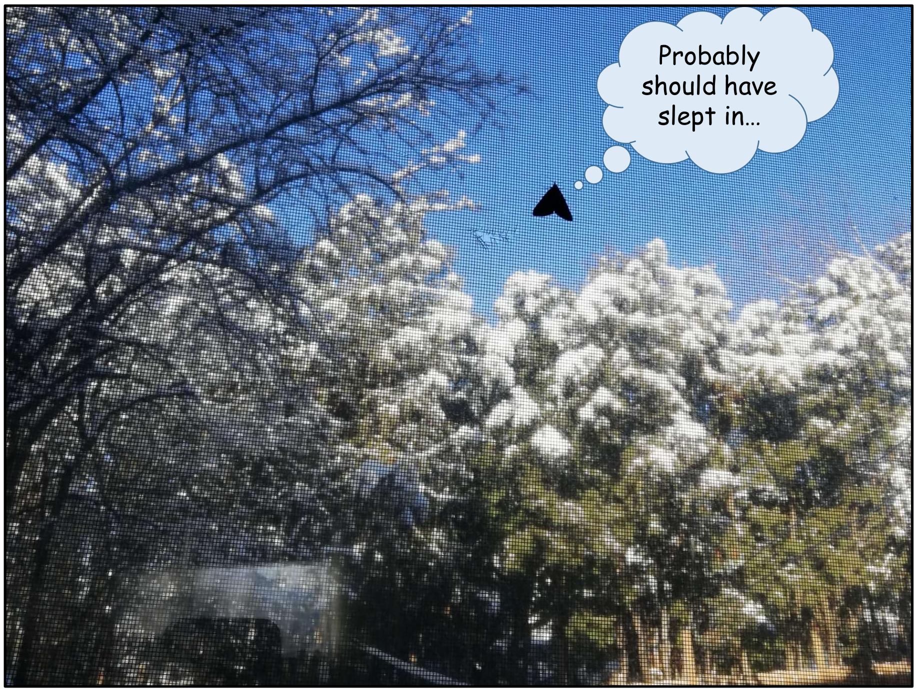


Hypena very clearly belongs to the second group in terms of its spatial behaviour, laying eggs singly, the larvae are very mobile, and it spreads its itself out thinly over the landscape. At our main release site, we see a bit of damage here, a bit there, scattered throughout the site. Hypena has moved across the street to the Fletcher Garden, but with its massive “ocean” of DSV, Hypena quickly gets diluted. Hypena may be there, but you don’t see it. We can only hope that even though it is spread so thinly that it is hard to spot now, but that the population is still growing and eventually it will rise to detectable levels on a wider scale. So best case scenario is that Hypena is hard to see for the time being, sprinkled through the landscape, worst case scenario is that its behaviour will preclude it from becoming an outbreak species and doing the job it was intended to do. Only time will tell…

Biocontrol takes time

Purple loosestrife *Lythrum salicaria* (left)
Photo taken summer 2000, Petrie Islands before the arrival of *Neogalerucella calmariensis* (above)

And biological control, unfortunately, takes the time it takes. Purple loosestrife is a good example. Biocontrol beetles introduced in 1992, wasn't until 20 years or so later the beetles began to be noticeably abundant. The hope is that Hypena might turn out to be like Neogalerucella the loosestrife beetle, and in a few years we will see outbreak densities of Hypena everywhere.



But until then, we must be patient.
(Hypena in the photo is an escapee from the Ottawa colony which was reared on my dining-room table last summer due to covid lab restrictions. It must have pupated under my sofa and has emerged way to early)

Thank you!

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Shane Minor

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Ottawa Field-Naturalists' Club